

## MICROSOMAL NUCLEOPROTEIN PARTICLES FROM PEA SEEDLINGS\*

By PAUL O. P. TS'O, PH.D., JAMES BONNER, PH.D., AND JEROME VINOGRAD, PH.D.

(From the Kerckhoff Laboratories of Biology and the Gates and Crellin Laboratories of Chemistry,† California Institute of Technology, Pasadena)

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Much progress has recently been made in the study of the ribonucleoprotein particles associated with the microsomal fraction of cell homogenates. The shape and size of these particles as well as their distribution and organization in animal tissues have been studied with the electron microscope (1-4). They appear as round particles with diameters of 100 to 300 Å in OsO<sub>4</sub>-fixed preparations of both sections of embedded pellets (5) and air-dried samples (6) previously treated with desoxycholate to remove attached membranes. The resuspended microsomes isolated from animal sources by differential centrifugation have been found to be heterogeneous in the ultracentrifuge (7) and to be made more homogeneous by treatment with desoxycholate (6). The particles in the microsomes of animal tissues are rich in pentosenucleic acid (5) and contain 40 to 50 per cent RNA on a dry weight basis (6). The same is true of the similar particles found in bacteria (8).

Less is known about microsomal particles from plant sources. Spherical particles 200 to 400 Å in diameter, but of uncertain chemical composition, have been found in fractions isolated from peanut cotyledons (9) and bean roots (10). The bean root particles have been reported to contain more than 15 per cent of nucleic acid (11).

*In vitro* studies of the mechanism of protein synthesis suggest that the microsomes are involved (6, 12). It is therefore, desirable to obtain purified and physicochemically well defined preparations of microsomal particles for further studies. The present paper reports the isolation and preliminary physicochemical characterization of microsomal particles from pea seedlings. The behavior of the isolated particles in the presence of various ions and under a variety of other conditions has also been investigated.

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### Materials and Methods

**Chemicals.**—The dipotassium salt of adenosinetriphosphate (Pabst) was used without further purification. The solution was neutralized to pH 7.0 with potassium hydroxide. Pancreatic ribonuclease (Armour) was used. All other chemicals were of reagent grade.

**Plant Tissues.**—Pea seedlings, *Pisum sativum*, were grown in vermiculite in the dark for 7 days at 25°C. The apical 2.5 to 4 cm. of the stems were collected. The tissue was ground in a mortar at 2–4°C. with washed sand and 0.4 M sucrose solution (0.5 ml. per gm. of tissue). The homogenate was filtered through cheese-cloth and centrifuged at 40,000 *g* (25,000 R.P.M.) for 10 minutes. The supernatant from this centrifugation will be termed the crude cytoplasmic extract. For experiments with pea roots seeds were germinated in a pan covered with damp cloth and paper.

**Chemical Analyses.**—The biuret analysis of Cornell, Bardawill, and David (13) was used for determination of protein. The analyses of total phosphorus was done by the Allen method (14). Total nitrogen was measured by the Nessler method of Lanni *et al.* (15). The semi-quantitative determination of desoxyribose was done by the diphenylamine method of Dische (16).

**Measurement of TCA-precipitable solids.**—Precipitation was carried out by adding 1 N TCA (trichloroacetic acid) in a volume ratio of 1:2. The precipitate of protein and nucleic acid was allowed to stand for a few hours at 0°C. and centrifuged and washed twice with 0.5 N TCA. The washed precipitate was dried to constant weight at 100°C.

**Ultracentrifugation.**—The preparative ultracentrifugation was performed in the No. 40 angle rotor in the model L ultracentrifuge, Specialized Instruments Company, Belmont, California. Analytical runs were performed in the model E instrument equipped with a temperature control system. The direction of sedimentation of all ultracentrifuge patterns is from right to left. Runs were performed at the speed of 35,600 R.P.M. unless stated otherwise.

**Viscosity.**—An Ostwald viscometer with a flow time for water of about 60 seconds and a shear gradient of about 700 sec.<sup>-1</sup> was used. All viscosity measurements were made at a temperature of 24.2 ± 0.05 C.

**Ultraviolet Spectroscopy.**—The UV spectrum of the microsome preparation was obtained with the Cary quartz spectrophotometer model 11 M, Applied Physics Corporation, Pasadena. The optical densities of other solutions were measured with a Beckman model DU spectrophotometer.

**Conductance Measurement.**—The conductivity of the solutions was measured with a portable A.C. electrolytic conductivity bridge manufactured by Leeds and Northrup Co., New York.

**Electron Microscopy.**—The electron microscope used was the model EMU-2A, Radio Corporation of America. The magnification was calibrated with carbon replicas of a grid containing 30,000 lines per inch supplied by Ernest F. Fullam, Inc., Pasadena. Microsome preparation (DC-2, see below) in water mixed with standard polystyrene latex was sprayed with a nebulizer onto metal grids coated with collodion. The samples dried in air rapidly. The screens were shadowed at an angle of about 4:1 with a gold-palladium alloy. The spherical polystyrene particles (2600 Å in diameter) serve as a check of the magnification as well as of the shadowing angle.

## RESULTS

### *Studies of Homogenates and the Isolation of Microsomal Particles*

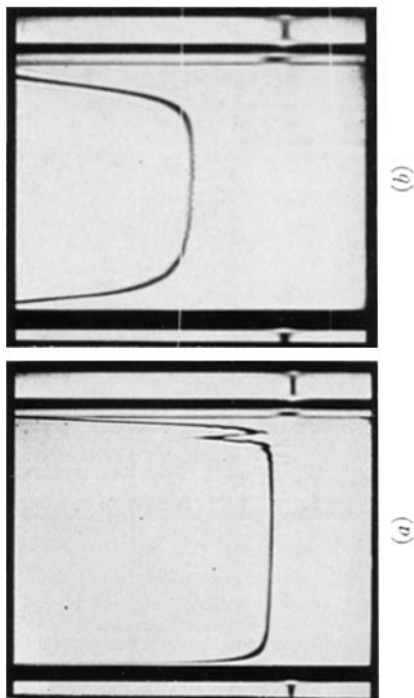
Since purified preparations of isolated microsomal particles were found to be sensitive to addition of electrolytes, the pH and the electrolyte concentration

of the whole homogenate were determined. The pH of extracts prepared with sucrose solution by the procedure described above was found to be always above 6 and usually 6.3–6.5. The conductance of such extracts is  $1.17 \times 10^{-3}$  mhos at 1°C., which corresponds to the conductance of a 0.015 M KCl solution. Taking into account the dilution of extract with the grinding medium, the presence of sucrose in 5 per cent concentration, and the fact that the average ionic mobility is lower than that of KCl, it is estimated that the pea epicotyl contains about 0.07 to 0.05 M electrolytes.

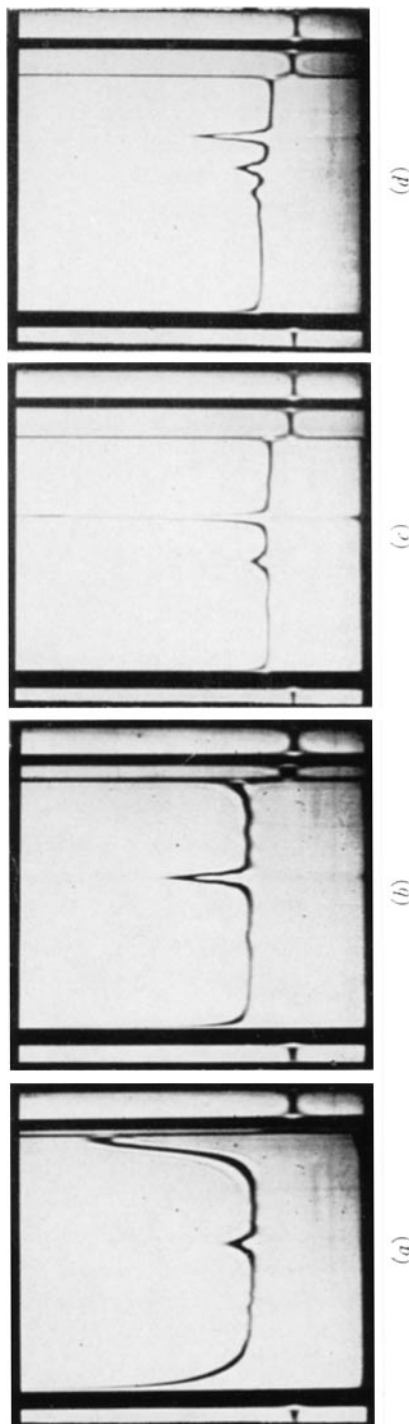
The distribution of particles in crude cytoplasmic extracts was first analyzed by ultracentrifugation. The patterns obtained at 25,980 R. P. M. and after very short sedimentation times are shown in Text-fig. 1 *a*. Two boundaries separate from the meniscus. The major boundary moves with a sedimentation coefficient of 74 and includes about 1 mg./ml. of material, based on a typical refractive index increment for protein. The minor component moves with a sedimentation coefficient of 110, as determined in the fractionated samples the flat base line ahead of these boundaries indicates that the crude extract does not contain much material with sedimentation coefficients between 200 and 300 S. After 25 minutes the speed was increased to 47,660 R.P.M. for 20 minutes. The patterns obtained at this period (Text-fig. 1 *b*) show a broadening of the unresolved boundary at the meniscus. These results indicate that there are no distinct groups of particles with sedimentation coefficients of 20 to 30 S. It is, therefore, concluded that most of the large particles in these extracts have sedimentation constants between 60 and 110 S with about 80 per cent of them having a value of 74 S.

The crude cytoplasmic extract was fractionated by the following procedure: The particles were sedimented from the crude extract at 110,000 *g* (40,000 R. P. M.) for 75 minutes. The pellet obtained from 11 ml. of the crude extract was resuspended in 2 to 4 ml. of distilled water with gentle but constant stirring for 1 hour. This solution was again centrifuged at 18,000 *g* (15,000 R. P. M.) for 10 minutes to remove undispersed aggregates. The supernatant fraction obtained from the first cycle of differential centrifugation is designated as microsomal particle preparation DC-1. The particles of DC-1 preparation were sedimented again at 110,000 *g* for 60 minutes. The pellet was resuspended in distilled water and the resultant suspension was again centrifuged at 40,000 *g* (25,000 R. P. M.) for 5 to 8 minutes for a final clarification. The solution containing particles purified by two cycles of differential centrifugation is designated as a DC-2 preparation of microsomal particles.

The ultracentrifugal patterns of the three above preparations, *i.e.* the original extract, the once centrifuged and resuspended pellet, and the DC-2 preparation, are compared with one another in Text-fig. 2 *a, b, c*. As the particles are progressively purified by differential centrifugation, the concentrations of 74 S and 110 S particles are increased while more slowly sedimenting material is



TEXT-FIG. 1. (a) Ultracentrifuge pattern of cytoplasmic extract from pea stems. Photograph obtained immediately after reaching speed of 25,980 R.P.M. Medium, *ca.* 0.2 M sucrose. Concentration, 4.7 mg./ml. (b) 20 minutes after reaching speed of 47,660 R.P.M.



TEXT-FIG. 2. (a) Ultracentrifuge pattern of cytoplasmic extract from pea stems. Medium, *ca.* 0.2 M sucrose. Concentration 3.3 mg./ml. (b) DC-1 preparation of microsomal particles in water. 3.5 mg./ml. (c) DC-2 preparation of microsomal particles in water. 4.0 mg./ml. (d) Same as (c) after 40 hours at 2°C.

removed as indicated by absence of material unresolved from the starting boundary in Text-fig. 2 *c*. From 300 gm. of fresh weight of pea epicotyl about 10 ml. of a clear DC-2 preparation containing 11 mg./ml. of TCA-precipitable material may be obtained. Area measurements of the ultracentrifuge diagrams from the foregoing clear solution and the crude extract together with data on volumes and TCA weights of preparation, indicate that about 30 per cent of the particles originally present in the cytoplasmic extract are recovered. Thus the TCA-precipitable material corresponds to 0.1 to 0.2 per cent of the pea epicotyl on a fresh weight basis and to 1 to 2 per cent on a dry weight basis.

TABLE I

*The Nucleic Acid Content of Fractions Obtained during the Purification of Microsomal Particles\**

	Per cent of total TCA-precipitable dry weight		
	Crude† cytoplasmic extract	Supernatant from 1st centrifugation. (110,000 g for 75 min.)	DC-2 preparation
TCA-precipitable phosphorus	0.53	0.015	3.4
	0.80	0.018	2.8
	0.93	0.022	2.9
			3.3
Ribose nucleic acid§	6	1.9	37
	9	2.1	30
	10	2.5	31
			37

\* Values obtained in separate experiments are given. Each value is an average of analyses performed in triplicate.

† The crude extract usually contains *ca.* 0.4 per cent TCA-precipitable material, one-quarter of which sediments in the first centrifugation.

§ Based on a conversion factor of 11 gm./gm. phosphorus.

The amount of TCA-precipitable phosphorus in the original extract, in the supernatant fraction from the first differential centrifugation, and in the DC-2 preparation of particles may be used as a preliminary measure of the nucleic acid content of the preparation. This information is summarized in Table I. The presence of a large amount of nucleic acid in the DC-2 preparation is confirmed by the ultraviolet spectrum.

The data in Table I show that most of the nucleic acid in the extract accompanies the particulate fraction and that after the particles have been sedimented, scarcely any nucleic acid remains in the supernatant. Comparison of data for the dry weight and the TCA-precipitable phosphorus in the crude extract and in the supernatant from the first centrifugation, shows that the sedimented material contains 25 to 30 per cent nucleic acid, in agreement with the composition for the DC-2 preparation.

*Some Physical and Chemical Properties of Pea Stem Microsomal Particles*

Ultracentrifugal analyses shows that the microsomal preparation consists of one major component (*ca.* 80 per cent) and four other minor components. The relative concentrations and sedimentation coefficients of each are given in Table II. Evidence given later indicates that components D and E can increase in concentration at the expense of component C, the major component. It is possible, therefore, that D and E arise from the breakdown of C. The preparations examined contained 0.3 to 0.5 per cent TCA-precipitable material, and showed in this range no variation of sedimentation coefficients with concentration. From the viscosity and electron optical data given below, it may be anticipated that the value of *S* for component C should be insensitive to con-

TABLE II  
*Sedimentation Coefficients and Relative Concentrations of Components in DC-2 Preparations of Microsomal Particles*

Component*	Sedimentation coefficient ( <i>S</i> <sub>20</sub> , Svedbergs)	Concentration per cent of total†		
		Exp. 147	Exp. 143	Exp. 148§
A	152 ± 6	2.3	3.7	1.2
B	110 ± 4	9.0	9.5	7.4
C	74 ± 1	79.0	80.5	83.0
D	60 ± 2	5.4	3.0	5.0
E	40 ± 2	4.2	3.2	3.0

\* Components are listed in order of their sedimentation coefficients.

† Corrected for the effect of radial dilution. Refractive index increments assumed to be the same.

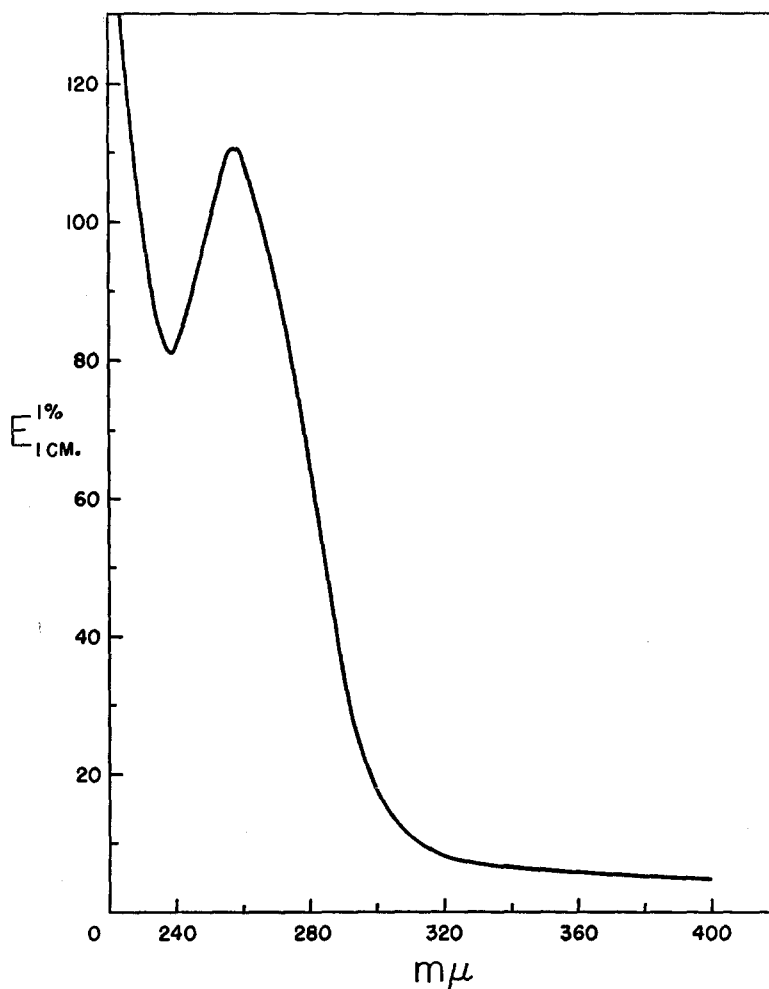
§ This sample contains 0.1  $\mu$  K maleate, pH 7.0. The electrolyte was added to the solution just before the run.

centration. No difference in sedimentation constant was detected when 0.1  $\mu$  maleate (pH 7) rather than water was used as the suspending medium. Thus values of 73.1 *S* and 75.0 *S* were obtained for component C in two experiments in which 0.1  $\mu$  maleate was used.

The absorption spectrum of the preparation of microsomal particles is given in Text-fig. 3 and the chemical composition of the preparation in Table III. The preparation contains 35 per cent nucleic acid and a small amount of phospholipide. The nucleic acid is of the ribonucleic acid type as no desoxyribose is detectable by the diphenylamine reaction.

The reduced viscosity was measured at a shear gradient of about 700 sec.<sup>-1</sup> In view of the low relative viscosity (1.02 to 1.05) and the low axial ratio of the particles as revealed by electron microscopy, the viscosity will be independent of the rate of shear in this range. The intrinsic viscosity was 12 cc./gm. for one preparation and 10 cc./gm. for a second as determined by extrapolation of

data obtained with distilled water solutions containing 0.2 to 1.0 per cent. Exposure of the particles to 0.1  $\mu$  maleate for a short time—15 minutes—lowered the value by less than 10 per cent.



TEXT-FIG. 3. The ultraviolet absorption spectrum of DC-2 preparation of microsomal particles. Medium, water, pH. 6.3.

The partial specific volume was computed from the composition of the preparation (Table III) and from assumed partial specific volumes of protein, 0.75, and ribonucleic acid, 0.578, (17) to be  $0.68 \pm 0.01$ . This estimate agrees with the value 0.67, obtained experimentally for turnip yellow mosaic virus which has a similar composition (18). Experimental measurements of the

partial specific volume of the microsomal particles were not undertaken because of the inability to carry out dialysis without formation of turbid solutions.

The viscosity coefficient for the microsomal particles calculated from the average intrinsic viscosity, 11 cc./gm., and the partial specific volume, 0.68, is 16.2, and corresponds to a frictional coefficient of 1.65 based on an assumed 40 per cent hydration. The gram molar particle weight of the major component C based on the sedimentation coefficient (Table II), the average

TABLE III  
*Composition of DC-2 Preparations of Microsomal Particles*

Component	Per cent of TCA-precipitable dry weight
Total nitrogen*	14 ± 0.5
Nucleic acid	
Estimated from extinction coefficient at 260 mμ†	31 38 34
Estimated from TCA-precipitable phosphorus§	31 30 37 37
Total protein	56 ± 2
Phospholipide¶	4.5

\* The total nitrogen was determined by Nessler's method.

†  $E_{1\text{ cm.}}^{1\%}$  of RNA taken to be 270 (19) and of protein 10.

§ These data are presented in Table I. The conversion factor from phosphorus to nucleic acid was taken to be 11.

|| The protein content was measured by the biuret method based on a standard of bovine serum albumin.

¶ The value of phospholipide was based on determinations of ether-ethanol-extractable phosphate. The conversion factor adopted was 24.

partial specific volume, and the average frictional coefficient of the whole preparation, is calculated to be  $4.5 \times 10^6 \pm 10$  per cent. The error here is based only on an estimate of individual errors in measurements performed in this work.

Electron micrographs of air-dried preparations are presented in Fig. 1. The particles approximate spheroids as they are circular and cast an ellipsoidal shadow. Measurements of shadow lengths and shadow angle show that the particles are flattened spheroids with heights of 170 to 200 Å. Data from measurements of fifty particles are given in Table IV. The particle diameters range from 220 to 350 Å, the majority being in the region of 280 Å. The particle



volumes in Table IV are calculated on the basis of the oblate spheroid model using 180 Å as the length of the minor axis. The gram molar particle weights in Table IV are computed on the assumption that the particles are solid and have a density corresponding to the previously adopted value, 0.68, for the partial specific volume.

#### *Stability of Microsomal Particles*

When DC-2 preparations are stored at 2°C. for 18 hours, little change in the ultracentrifuge pattern can be detected. If storage time is lengthened to 40 hours, the solution becomes turbid and component A, 152 S, is increased in concentration by a factor of 2-4 at the expense of component C, 74 S (Text-fig. 2 *d*). The aggregation process that occurs on storage leads to resolvable aggregates, such as component A which from its sedimentation coefficient appears to be a dimer of component C, and to unresolvable aggregates as indicated by the

TABLE IV  
*Dimensions and Computed Weights of Individual Microsomal Particles in Electron Micrographs\**

Diameter	Particle volume	Gm. molar particle weight	No. of particles inside the range
A	$A^3 \times 10^{-6}$	$1 \times 10^{-6}$	
220-250	4.2-5.3	4.0-4.7	23
280-310	6.7-8.2	6.0-7.3	22
330-350	9.3-10.5	8.3-9.4	5

\* Fifty particles selected at random from the field were measured.

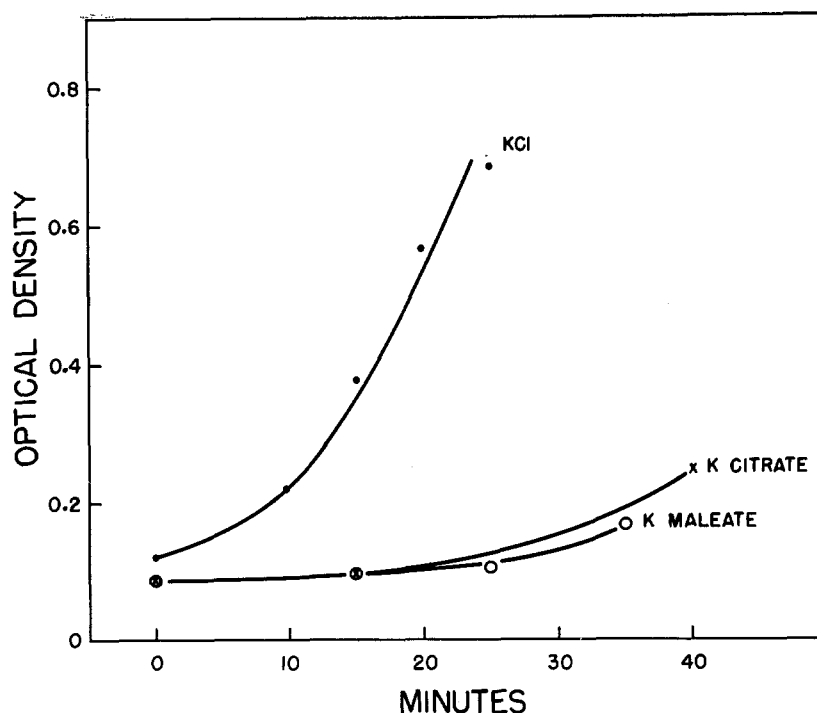
elevated base line ahead of the fastest boundary. Upon dialysis for 10 hours in distilled water (pH 5.9) the DC-2 preparations become turbid, and all the 74 S particles clump together to form rapidly sedimenting and non-resolvable aggregates.

Addition of 25 µg. of RNAase to a 0.5 per cent DC-2 preparation at room temperature causes the solution to become turbid within half an hour. The pH is simultaneously lowered from 6.3 to 5.1 presumably the result of the liberation of phosphate groups. Ultracentrifugal analyses again showed the presence of large aggregates which sediment rapidly without resolving.

The DC-2 preparations are labile in the presence of electrolytes. 10 to 20 minutes after the addition of 0.2 µmole/ml. of MgSO<sub>4</sub>, pH 7, the solution becomes turbid and the ultracentrifuge pattern showed a tilted base line and no boundaries. At 1 µmole/ml. of MgSO<sub>4</sub> cloudy precipitates appear in the solution immediately. Two µmole/ml. of MgCl<sub>2</sub> or of CaCl<sub>2</sub> pH 7.0, also renders the solutions turbid.

The increases of turbidity with time in the presence of various potassium salts at 0.1 ionic strength at room temperature are given in Text-fig. 4, which

shows that KCl, pH 6.5, acts more rapidly than K maleate or K citrate, pH 7.0. The ultracentrifuge patterns obtained on samples to which K maleate was added 15 minutes earlier are indistinguishable in regard to sedimentation coefficients and relative areas of the different components from patterns obtained with samples in distilled water. The turbidity of the solution at this time, as shown in Fig. 4 is substantially unchanged. On the other hand, 2 hours after addition of K maleate the relative concentrations of component D (60 S) and

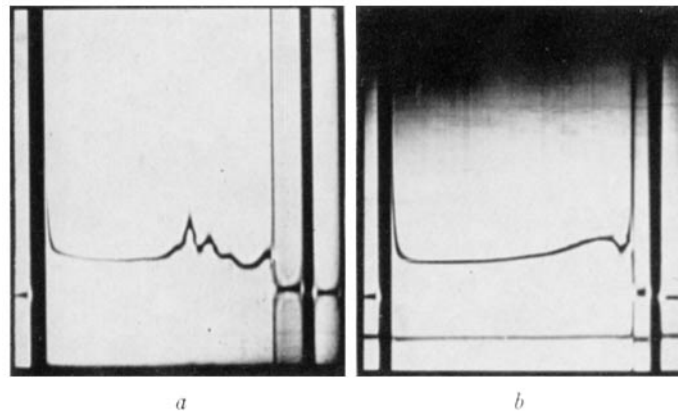


TEXT-FIG. 4. Optical density of microsomal particle solutions after addition of various salts. Ionic strength, 0.1  $\mu$ , wave length, 400 m $\mu$ .

component E (40 S) are increased by 3 to 5 times, the base line ahead of component C is elevated, and the concentration of component C is decreased (Text-fig. 5 *a*). These results suggest that both degradation and aggregation of the particles have occurred. The particles of component C appear to break down to components D and E, which sediment more slowly. The presence of D (2 to 4 per cent) and E (2 to 4 per cent) in the initial DC-2 preparations may be due to the breakdown of C during the isolation of the particles. Random aggregation leading to an elevation of the base line also occurs. This accounts for the increased turbidity.

The effect of ATP on the particles is quite different from that of the neutral

salts described above. The addition of ATP, pH 7, at a concentration of 2 to 3  $\mu\text{mole/ml.}$ , decreases the turbidity of the microsome solution. The optical density at 400  $\text{m}\mu$  at which neither ATP nor microsomes absorb markedly, was reduced by ATP in one case from 0.31 to 0.16 and in another case from 0.18 to 0.10. This suggests that ATP causes a breakdown of the particles with little aggregation, as is shown in the ultracentrifuge pattern (Text-fig. 5 *b*) in which only a slow and rapidly spreading boundary is visible. Precipitates formed by addition of 0.5  $\mu\text{mole/ml.}$   $\text{MgSO}_4$  dissolved readily upon addition of 2 to 3  $\mu\text{mole/ml.}$  of ATP.

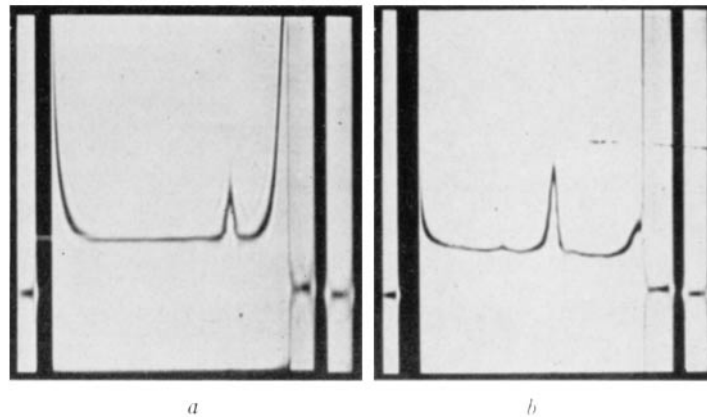


TEXT-FIG. 5. (*a*) Ultracentrifuge pattern of DC-2 preparation 2 hours after addition of 0.1  $\mu$  K maleate, pH. 7.0. Concentration, 4.0  $\text{mg./ml.}$  (*b*) 20 minutes after addition of 5  $\mu\text{mole/ml.}$  of ATP, pH. 7.0. 4.0  $\text{mg./ml.}$

#### *Studies of Particles in Roots of Pea Seedling*

A preliminary investigation has been made of the fast sedimenting particles of the roots of 3-day-old pea seedlings. 50 gm. of fresh roots, *ca.* 1.5 cm. long, were ground in a mortar with addition of 50 ml. of 0.4 M sucrose and 0.01 M potassium phosphate, pH 6.9. The homogenate was then filtered through cheese-cloth and clarified by centrifugation at 15,000  $g$  for 10 minutes to remove nuclei, mitochondria, and debris. Text-fig. 6 *a* shows the composition of this crude extract as analyzed in the ultracentrifuge. Only one major boundary with a sedimentation coefficient of  $66 \pm 3$  S was present. This extract was then centrifuged at 110,000  $g$  for 60 minutes and the pellet redissolved in the sucrose-phosphate medium. Few of the particles went back into suspension in the extraction medium. Furthermore, the suspension obtained contained a series of aggregates and two to three major components rather than the one major component originally present. Subsequent investigation revealed that the aggregation is due to the presence of phosphate and that when phosphate is

absent, the particles resuspend readily. The suspended pellet also contained but one major component as did the original extract. Further study showed that neither sucrose nor salts are essential components of the extraction medium. Material extracted from the pellet by 0.05 M KCl, Text-fig. 6 *b*, gave essentially the same ultracentrifuge pattern as material extracted by 0.3 M sucrose. This suggests that the microsomal particles do not possess a membrane as do the mitochondria and may be insensitive to the osmotic concentration of the extraction medium.



TEXT-FIG. 6. (*a*) Ultracentrifuge pattern of cytoplasmic extract from pea roots in *ca.*  $5 \times 10^{-3}$  M potassium phosphate, pH. 7.0, and 0.2 M sucrose. Concentration, 3.5 mg./ml. (*b*) DC-1 particles in 0.05 M KCl. Concentration, 3.5 mg./ml.

#### DISCUSSION

That the microsomal particles here described possess significance for the plant may be judged from the facts that they contain at least 60 to 80 per cent of the cytoplasmic RNA, (RNA not sedimented by 40,000 R. P. M. in 10 minutes in the No. 40 rotor) and that the percentage of RNA in the particles is high, 31 to 37 per cent.

The microsomal particles contain only small amounts of phospholipide as shown by the low content of ether-hot-ethanol-extractable phosphate as well as by the agreement between the nucleic acid content as measured by TCA-precipitable phosphate and by spectroscopy. The high nitrogen content of the particles suggests that the amount of other materials low in nitrogen, such as fatty acids and carbohydrate, must also be small, and that we are dealing here with what is essentially a nucleoprotein.

The DC-2 preparations appear to contain only two ultracentrifugally resolvable components, the C component, 80 per cent, and the B component, 9 per cent. The rest of the material probably arises from the C component during

preparation. The data suggest that the A component of the original extract may be the dimer of C component and the D and E components may be breakdown products of C. Under the electron microscope, about 90 per cent of the particles possess diameters of 240 and 300 Å while 10 per cent possess diameters of 340 Å in an air-dried preparation. The particles are well separated except at the edge of a droplet. The reason for the spread in particle size between 240 and 300 Å in diameter is not known. It may be an artifact of the air drying procedure or may be due to the limited accuracy of the measurements.

The chemical composition of the present particles is in contrast to that of animal microsomes not treated with deoxycholate, but is closely similar to that of the nucleoprotein particles. The animal microsomes contain 5 to 10 times more phospholipide and about 50 per cent less nucleic acid than the present particle preparation. These facts together with the fact that 80 per cent of the present particles move with one ultracentrifugal boundary and that the particles appear discrete in the electron microscope, suggest that the present preparations contain very little of the membrane component associated with the nucleoprotein in the animal microsomes. Whether or not the plant particles are associated with an equivalent of the endoplasmic reticulum in the living cell is not yet known.

The present particles have been designated microsomal particles because of the resemblance in sedimentation coefficient and nucleic acid-protein ratio to particles (5-7) that have been isolated from animal microsomes. These microsomal particles are not to be confused with other nucleoproteins that may be obtained from plant sources such as fraction I (20) from green leaves.

Though the intrinsic viscosity of preparations of the present particles is low, it is still higher than the values expected from the shape of the particles as observed by electron microscopy. This may be the result of the electroviscous effect, a large internal hydration, the presence of aggregated particles, or other viscous impurities present in small amounts.

The gram molar particle weight of the major component has been estimated by two methods. The value based on intrinsic viscosity, partial specific volume, and sedimentation coefficient is 4.5 million  $\pm$  10 per cent compared with 4 to 6 million from electron microscopy. About 10 per cent of the particles in the electron micrographs appear to have a weight of 7 to 9 million and these may correspond to the ultracentrifuge component B of 110 S.

#### SUMMARY

Ultracentrifugal analysis of an extract of pea epicotyls, previously freed of debris and larger particles by centrifugation at 40,000 *g* for 10 minutes, has revealed the presence of a major component which possesses a sedimentation coefficient of 74 S. This component constitutes about 25 per cent of the TCA-precipitable material in the clarified epicotyl extract and is estimated to make

up 1 to 2 per cent of the dry weight of the original tissue. In size, chemical composition, and morphology, the 74 S component resembles the nucleoproteins of the microsomes from animal tissues.

The 74 S component of pea epicotyl extracts has been purified by repeated cycles of differential centrifugation to yield a preparation which is 80 per cent homogeneous in the analytical ultracentrifuge. It has been found to contain 30 to 37 per cent RNA as judged by a variety of analytical techniques. Approximately 55 per cent of the weight of the material is protein and a further 4.5 per cent phospholipide.

Electron micrographs of air-dried specimens of the purified preparation show the 74 S constituent to be flattened spheres with an average height of 180 Å and an average diameter of approximately 280 Å. The molecular weight of the 74 S particles is computed from sedimentation, viscosity, and partial specific volume data to be 4.5 million  $\pm$  10 per cent in agreement with the value estimated from electron micrographs.

The 74 S or microsomal component of pea epicotyls is rapidly aggregated in the presence of low concentrations of Mg ions or by somewhat higher concentrations of Ca or K salts. ATP on the contrary causes resolution of electrolyte-induced microsomal aggregates with simultaneous degradation of the particles to an ultracentrifugally inhomogeneous mixture of lower molecular weight materials.

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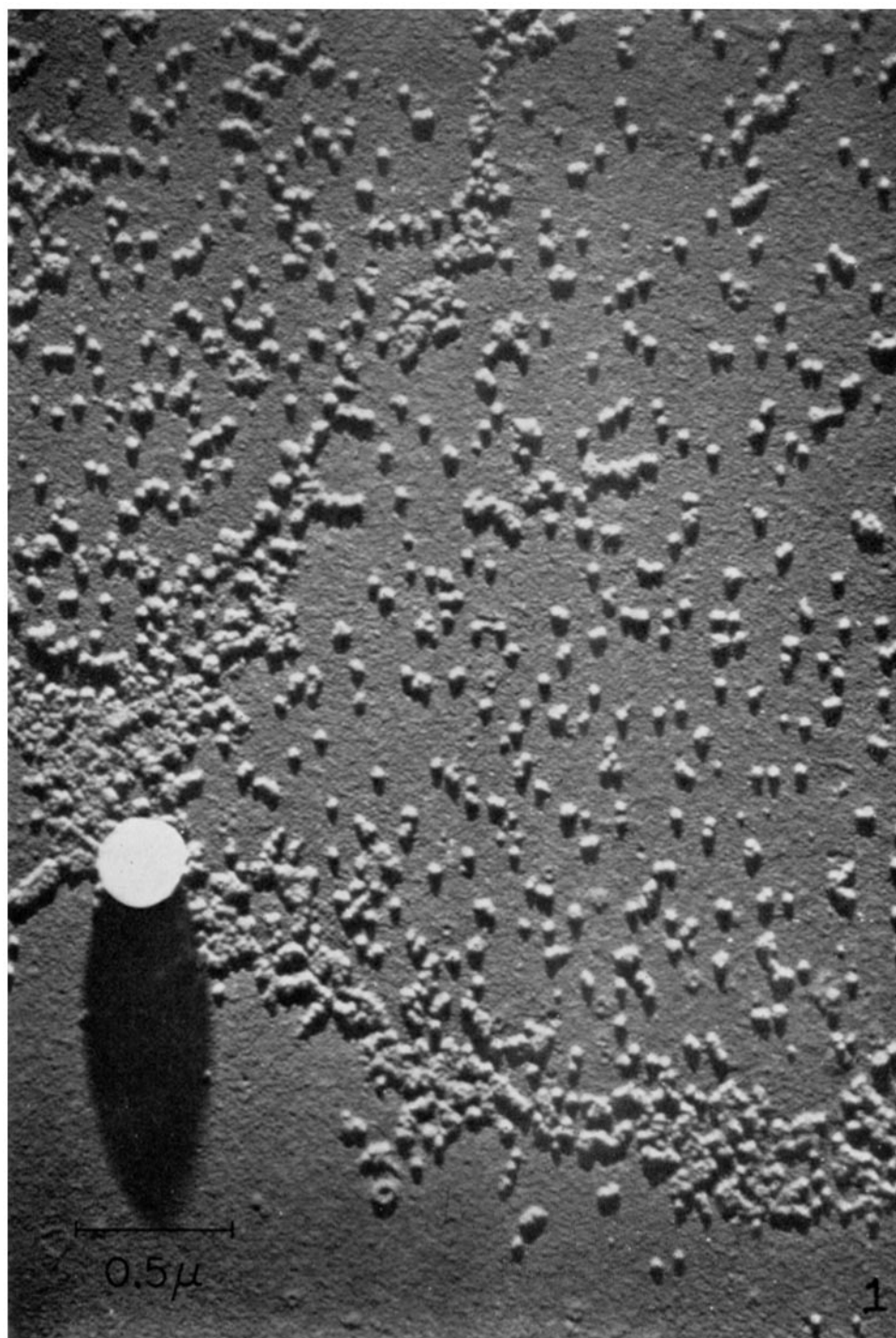
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## EXPLANATION OF PLATE 124

FIG. 1. The electron micrograph of pea stem microsomal particles. Magnification, 44,000.





(Ts'o, Bonner, and Vinograd: Microsomal nucleoprotein particles)